The Journal of Basic & Applied Zoology (2015) **70**, 16–24

HOSTED BY

ELSEVIER

The Egyptian German Society for Zoology

The Journal of Basic & Applied Zoology

www.egsz.org
www.sciencedirect.com

Histological and histochemical characterization on stomach of *Mystus cavasius* (Hamilton), *Oreochromis niloticus* (Linnaeus) and *Gudusia chapra* (Hamilton): Comparative study

Saroj K. Ghosh, Padmanabha Chakrabarti *

Fisheries Laboratory, Department of Zoology, The University of Burdwan, Burdwan 713104, West Bengal, India

Received 18 August 2014; revised 23 March 2015; accepted 1 April 2015
Available online 26 April 2015

KEYWORDS

Histoarchitecture;
Histochemical nature;
Stomach;
Mystus cavasius;
Oreochromis niloticus;
Gudusia chapra

Abstract The histological features and histochemical characterization of the stomach were investigated in *Mystus cavasius* (Hamilton), *Oreochromis niloticus* (Linnaeus) and *Gudusia chapra* (Hamilton) having different feeding habits. Histologically the stomach of all the three fishes was made up of mucosa, submucosa, muscularis and serosa. The mucosa of superficial epithelium consists of a single layer of compactly arranged columnar epithelial cells. Prominent gastric glands are present in *M. cavasius* whereas in *G. chapra* the gastric glands are totally absent in the gizzard like stomach. However, in *O. niloticus* tubular gastric glands are present in the glandular epithelium of caecal like stomach. The distribution and chemical nature of mucopolysaccharides in the aforementioned fishes were studied histochemically by employing Periodic Acid Schiff's in combination with the Alcian Blue (PAS–AB) technique. Columnar epithelial cells lining the mucosa of the stomach including mucosal border were provided with exclusively neutral mucin which was probably involved in the protective functions against acid and enzymes. The different intensities of reaction of Best Carmine (BC) for glycogen in the epithelial lining and gastric glands of the stomach of the aforesaid three fish species under study were discussed. The intense reaction for protein and tryptophan was noticed in the gastric epithelium and gastric glands of *M. cavasius* probably due to accumulation of zymogen granules in the gastric glands. On the contrary, moderate reaction for protein and tryptophan was associated with the epithelial cells and gastric glands of *O. niloticus* and *G. chapra*. The cytoarchitecture and different degrees of localization of mucopolysaccharides, glycogen, protein and tryptophan in the stomach of *M. cavasius*, *O. niloticus* and *G. chapra* were correlated with the functional significance of the region concerned.

* Corresponding author at: Department of Zoology, The University of Burdwan, Golapbag, Burdwan 713 104, West Bengal, India. Tel.: +91 342 2634798; fax: +91 342 2657938.
E-mail address: dr.pachakrabarti@gmail.com (P. Chakrabarti).

Peer review under responsibility of The Egyptian German Society for Zoology.

http://dx.doi.org/10.1016/j.jobaz.2015.04.002

Introduction

The stomach of teleosts is well developed and structurally adapted to accommodate a wide variety of diets (Reifel and Travill, 1978). In fact, each species has its own structural adaptations to the stomach towards its specific food habits which varies greatly in regard to the percentage of animal and plant food materials. Kapoor et al. (1975) reported that the stomach is absent in a number of fish species, probably depending on the taxonomic position, not on feeding habits. When present, the stomach is the portion of the alimentary canal most subject to gross variations. Fish gastric histology is generally simpler than that of higher vertebrates in that the gastric glands contain only one cell type that secretes both pepsinogen and hydrochloric acid (Rebolledo and Vial, 1979; Gallagher et al., 2001; Diaz et al., 2003).

The teleost stomach shows a morphology which exhibits a distinct difference on that correlates with diet, feeding habit, body shape and also environmental conditions (Anderson, 1986; Winemiller et al., 1995). A very special adaptation is the modification of the stomach into a masticatory apparatus as reported by Pillay (1953) in *Mugil tade*. Kapoor (1957) advocated gizzard like stomach in *Gudusia chapra* and Khanna (1961) noticed muscular infoldings in *Tenualosa ilisha* and *Mugil corsula*. The presence of mucosubstances in the gastric epithelial cells has been observed in most of the teleosts (Kapoor et al., 1975; Gona, 1979; Murray et al., 1994). However, the literature containing the detection of acid and neutral mucopolysaccharides in the fish stomach and their significance have been rarely reported (Kazorić et al., 2007; Ikpegbu et al., 2013). On the contrary, localization and distribution of glycogen, protein and tryptophan contents in the various cellular elements in the stomach and their role in the digestion process in teleosts are few (Arellano et al., 2001; Carrassón et al., 2006).

However, no attempt has been made to correlate the functional significance of various cells in the teleostean stomach in relation to their feeding habits. The aim of the present work is to assess the histoarchitecture and the chemical nature of mucins, localization and distribution of glycogen, protein and tryptophan content in the stomach of *Mystus cavasius* (Siluriformes, Bagridae), *Oreochromis niloticus* (Perciformes, Cichlidae) and *G. chapra* (Clupeiformes, Clupeidae) having different feeding habits. This study no doubt, would help to get ideas regarding the structural detailed and precise chemical constitution of various cells of the stomach of the selected food fishes of India.

Materials and methods

Adult mature specimens of *M. cavasius* (10–12 cm in total length) and *O. niloticus* (18–20 cm in total length) were procured from the local freshwater body of Burdwan and *G. chapra* (7–9 cm in total length) were collected from the river Ganga near Dhatrigram, Burdwan, West Bengal, India. The fishes were sacrificed by decapitation following the guidelines given by the Institutional Ethics Committee and the alimentary canal was dissected out from the body. The stomach portion of experimental fishes was dissevered, cut into small pieces and fixed in aqueous Bouin's fluid and 10% neutral formalin for 18–20 h. After dehydration in a graded series of ethanol the fixed tissues were cleared with xylene and embedded in 56–58° C paraffin wax for histological study and 52–54° C paraffin wax for histochemical studies. Sections for histological studies were cut at 3–4 μm thickness using a rotary microtome and stained with Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain (Mallory, 1936). For histochemical tests, 8–10 μm paraffin sections were cut and subsequently subjected to the following histochemical techniques:

Periodic Acid Schiff's (PAS) in combination with Alcian Blue (AB) (PAS–AB) method for detection of neutral and acid mucins (Mowry, 1956)

Deparaffinized sections were brought to distilled water through downgraded ethanol series and oxidized in 1% aqueous periodic acid solution for 10–15 min at room temperature. After washing in distilled water, the sections were immersed in Schiff's reagent for 45 min and washed again in water. The sections were then immersed in 1% AB (8 GX, Sigma) in 3% acetic acid (pH 2.5) for 45 min. Then the slides were finally washed in running tap water, dehydrated through ascending series of ethanol, cleared in xylene and mounted in DPX.

Best's Carmine (BC) method for detection of glycogen (Best, 1906)

The dewaxed sections were passed into absolute ethanol and placed in 1% celloidin in equal parts of absolute ethanol and ether for 2 min. Celloidinized slides were transferred to distilled water and stained with carmine solution (Carmine stock solution – 15 ml, concentrated Ammonia – 12.5 ml and methanol – 12.5 ml) for 45 min. The stained slides were then differentiated in Best's differentiator (methanol – 40 ml, ethanol – 80 ml and distilled water – 100 ml), washed in 80% ethanol, dehydrated in absolute ethanol. Then the slides were placed in equal volume of absolute ethanol and ethyl ether mixture to remove the celloidin layer. The sections were cleared in xylene and mounted in DPX.

Mercury Bromophenol Blue (MBPB) method for detection of basic proteins (Mazia et al., 1953)

Deparaffinized sections were brought to distilled water and immersed in a freshly prepared MBPB solution (0.4 g $HgCl_2$ in 40 ml 2% acetic acid and then added 20 mg Bromophenol blue) for 2 h at room temperature. After rinsing in 0.5% acetic acid for 5 min, the sections were transferred directly to the tertiary butyl alcohol overnight. Then the slides were cleared in xylene and mounted permanently in DPX.

Dimethylaminobenzaldehyde (DMAB)-Nitrate method for detection of Tryptophan (Adams, 1957)

Deparaffinised sections were removed from absolute ethanol and coated with a thin film of celloidin (0.5%). Then the

sections were immersed in 5% *p*-dimethylaminobenzaldehyde in concentrated HCl. The sections were transformed to 1% solution of $NaNO_2$ in concentrated HCl for 1 min., washed in tap water and differentiated in 1% acid alcohol. The slides were dehydrated through ascending series of ethanol, cleared in xylene and mounted in DPX. Staining slides were examined and photographed under Olympus-Tokyo PM-6 compound microscope.

Results

Histology

M. cavasius

The stomach of *M. cavasius* is U-shaped; it is divisible into anterior cardiac and posterior pyloric portions. The mucosa of the cardiac stomach consists of surface and gastric epithelium. The surface epithelium is made up of a single layer of columnar epithelial cells and the gastric epithelium consists of gastric glands (Fig. 1A and B). The lamina propria, in the form of connective tissue network lies in between the gastric glands. The submucosa is made up of thick fibres of connective tissue innervated with blood vessels (Fig. 1A and B). The muscularis consists of the outer longitudinal and an inner thick circular muscle layer (Fig. 1A). Histologically, the pyloric stomach is similar to the cardiac stomach except that the mucosa is without gastric glands and circular muscle layer is thick.

O. niloticus

The stomach of *O. niloticus* is of caecal type. The wall of the stomach is made up of the innermost mucosa, submucosa, muscularis and outermost serosa (Fig. 1C). The mucosa is made up of superficial and glandular epithelium. The superficial epithelium is composed of compactly arranged single layer of columnar epithelial cells. The glandular epithelium consists of tubular gastric glands which are made up of rhomboidal shape of cells (Fig. 1D) with spherical nucleus in each cell. The submucosa is well vascularized and made up of connective tissue network. There are no gastric glands in pyloric region. The muscularis of the stomach has an inner circular and outer longitudinal layer. Serosa layer is distinct (Fig. 1C).

G. chapra

A true stomach is absent in *G. chapra* and in its place a greatly thickened gizzard like stomach is present. The mucosa of the stomach is thrown into short and stubby villi. The mucosa is lined with a single layer of compactly arranged comparatively stubby columnar epithelial cells which are almost equal in size. Rounded or oval type of gastric type of glands is present in this region (Fig. 1F). The lamina propria is made up of connective tissue and communicates with the submucosa. The submucosa is thick being composed of connective tissue and blood vessels in between. The muscularis layer is composed of an inner extremely thick circular muscle layer (Fig. 1E) and an outer thin longitudinal muscle layer. Serosa layer is very distinct.

Histochemistry

Detection and localization of mucopolysaccharides

The combined PAS–AB test imparts purple colour due to PAS for neutral mucin and bright blue colour for AB reaction due to the presence of acid mucin exclusively. In the present investigation the columnar epithelial cells of gastric mucosa of *M. cavasius*, *O. niloticus* and *G. chapra* show intense purple colour to PAS reaction but negative to AB suggesting the presence of exclusively neutral mucosubstances (Fig. 2A, C and D). The gastric glands of *M. cavasius* and *O. niloticus* also reacted moderately with PAS (Fig. 2A and B) whereas the tubular neck cells of *G. chapra* impart intense PAS reaction (Fig. 2C and D). The connective tissue of submucosa and lamina propria of all the three fish species shows purple-bluish colour with this test suggesting the presence of neutral and acid mucopolysaccharide content (Fig. 2A, B and D). The muscularis layers produce negative reaction to this combined test.

Detection and localization of glycogen

Best's carmine test indicates different intensities of reaction of glycogen content in the columnar epithelial cells, gastric glands and connective tissues of submucosa and lamina propria of *M. cavasius*, *O. niloticus* and *G. chapra*. However, maximum glycogen reaction is discernible in the prominent columnar epithelial cells lining the gastric epithelium of *M. cavasius* and *O. niloticus* (Fig. 3A and B). Moderate reaction of glycogen is found to occur in the epithelial cells of *G. chapra* (Fig. 3C and D). Intense content of glycogen is present in gastric glands of *M. cavasius* (Fig. 3A) and tubular gastric glands of *O. niloticus* (Fig. 3B) while the inner lining of the tubular gastric glands of *G. chapra* exhibits moderate reaction (Fig. 3C and D). Weak reaction of glycogen is observed in the connective tissue of submucosa and lamina propria of all the experimental fishes under study (Fig. 3A, B and D).

Detection and localization of protein

All the four histological layers of the stomach react positively for protein histochemical test with varying intensities in accordance with their protein content (Fig. 4A, C and D). The columnar epithelial cells of gastric epithelium in *M. cavasius* and *O. niloticus* exhibit intense reaction with MBPB (Fig. 4A and B) while weak reaction to protein is discernible in the columnar epithelial cells of *G. chapra* (Fig. 4D). The gastric glands of *M. cavasius* and *O. niloticus* appear as a deep blue colour with protein reaction (Fig. 4A–C) while intense reaction for the same is detected in the tubular glands of the stomach of *G. chapra* (Fig. 4D). Moderate to weak protein reaction is associated with the connective tissue of submucosa and lamina propria in *M. cavasius*, *O. niloticus* and *G. chapra* (Fig. 4A, C and D). The muscularis layer and blood vessels furnish somewhat higher intensity of reaction for protein material in the aforesaid fish species (Fig. 4A, B and D).

Detection and localization of tryptophan

The columnar epithelial cells of gastric mucosa exhibit moderate to weak reaction with DMAB in *M. cavasius* (Fig. 5A), *O. niloticus* (Fig. 5C) and *G. chapra* (Fig. 5D). The gastric glands

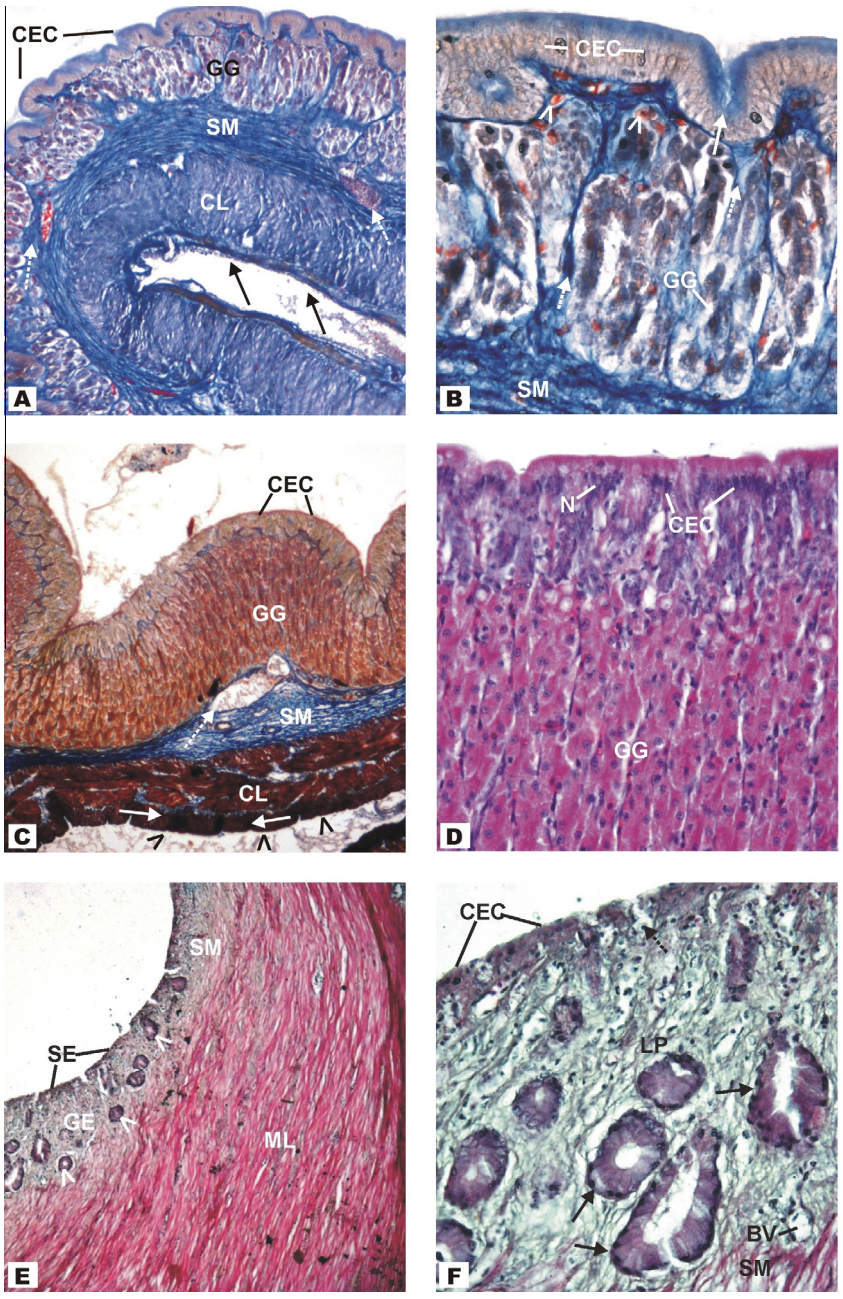

Figure 1 Photomicrographs of section of the stomach of *M. cavasius*, *O. niloticus* and *G. chapra* showing histological architecture stained with Delafield's Haematoxylin – Eosin (HE) and Mallory's triple (MT) stain: (A) stomach of *M. cavasius* exhibiting mucosa with a single layer of columnar epithelial cells (CEC) and gastric glands (GG). Note the presence of submucosa (SM) with blood vessels (BV) (broken arrows), outer longitudinal muscle layer (solid arrows) and inner circular muscle layer (CL) (MT) × 100, (B) surface epithelium of *M. cavasius* showing a single layer of compactly arranged CEC. Note prominent gastric cells in gastric glands (GG) separated by lamina propria (LP) (broken arrows). Note the presence of submucosa (SM) and blood cells (arrow heads) in LP. Solid arrow indicates crypt of surface epithelium (MT) × 400, (C) stomach of *O. niloticus* showing mucosa with tightly packed CEC and GG, submucosa (SM) with BV (broken arrow), inner circular muscle layer (CL), outer longitudinal muscle layer (solid arrows) and serosa (arrow heads) (MT) × 100, (D) stomach of *O. niloticus* showing surface epithelium with compactly arranged CEC with prominent nuclei (N) and extended layer of tubular gastric glands (GG) (HE) × 400, (E) surface epithelium (SE) with rugae like villi and gastric epithelium (GE) with tubular structure (arrow heads) of the stomach of *G. chapra*. Note thick muscularis layer (ML) and thin submucosa (SM) (HE) × 100, (F) higher magnification of the stomach of *G. chapra* showing short columnar epithelial cells (CEC). Note connective tissue network of LP and tubular glandular structure (solid arrows) in LP. Note also crypts of the stomach wall (broken arrow) and blood vessels (BV) in submucosa (SM) (MT) × 400.

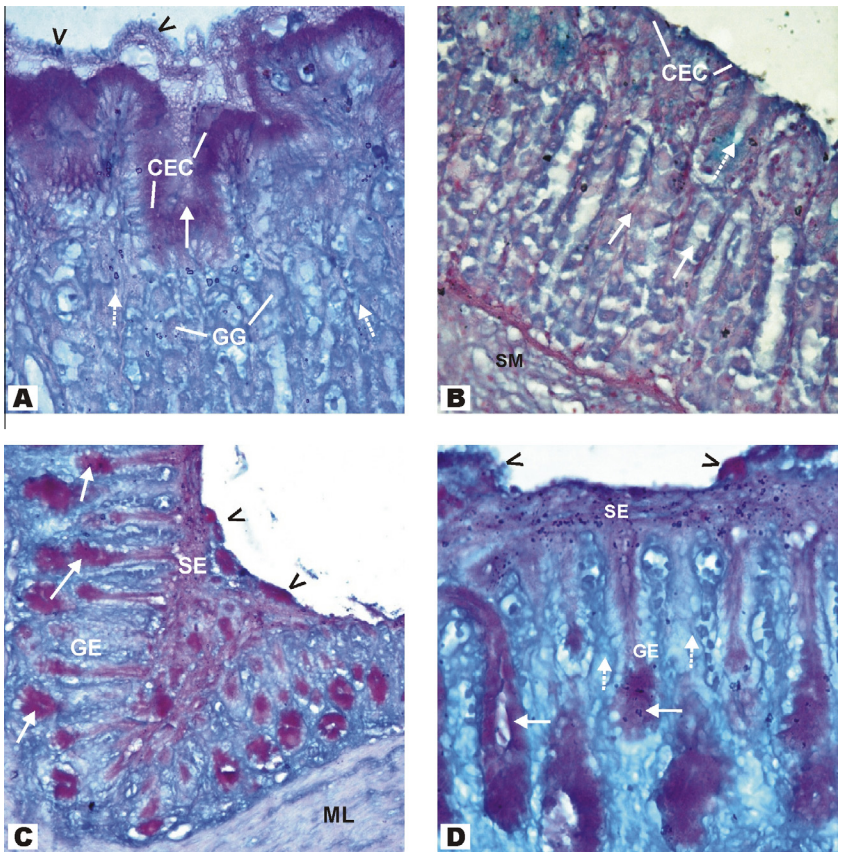

Figure 2 Photomicrographs of section of the stomach of *M. cavasius*, *O. niloticus* and *G. chapra* showing Periodic Acid Schiff's (PAS) in combination with Alcian Blue (AB) (PAS–AB) reaction: (A) showing intense localization of neutral mucins in the columnar epithelial cells (CEC) including the crypt region (solid arrow) and secreted luminal mucin (arrow heads) in the stomach of *M. cavasius*. Note moderate reaction of PAS in gastric glands (GG) and lamina propria (LP) (broken arrows) (PAS–AB) × 400, (B) intense PAS reaction in CEC in the stomach of *O. niloticus*. Note moderate reaction in GG (solid arrows). Broken arrow indicates crypt of the stomach wall. SM denotes submucosa (PAS–AB) × 400, (C) intense purple colour of neutral mucin in the surface epithelium and tubular glandular structure (solid arrows) of *G. chapra*. Note intense reaction in secreted luminal mucin (arrow heads). ML denotes muscularis layer showing feeble reaction (PAS–AB) × 200, (D) higher magnification of the stomach of *G. chapra* showing intense PAS reaction in surface epithelial layer (SE) and secreted luminal mucin (arrow heads). Note intense reaction in tubular glands (solid arrows) and weak reaction in LP (broken arrows) (PAS–AB) × 400.

of *M. cavasius* appear as a deep blue granular structure with DMAB test indicating the presence of tryptophan containing zymogen granules (Fig. 5A and B). In the stomach of *O. niloticus* and *G. chapra* however, the lining of the tubular gastric glands and tubular glandular structure are highly reactive to this test confirming the presence of a considerable amount of ergastic substances therein (Fig. 5C and D). The submucosal connective tissue and muscularis layers react moderately to this test in all the fish species studies (Fig. 5A, C and D).

Discussion

The digestive tract of teleost is well adapted to modes of feeding and kinds of diet. The alimentary canal of fish exhibits a remarkable diversity of morphological and functional characteristics (Murray et al., 1996; Buddington et al., 1997; Banan Khojasteh, 2012; Khalaf Allah, 2013). In fact, each fish species has its own structural adaptations of the stomach towards its specific food habit. In the present investigation a comparative account of the stomach of *M. cavasius*, *O. niloticus* and *G. chapra* has revealed many variations that are undoubtedly correlated with their different feeding habits. In *M. cavasius* the stomach is U-shaped and probably allow for stretching during food consumption involved in carnivorous mode of feeding. *O. niloticus* being a herbivorous feeder requires space and retention of food for effective acid hydrolysis thus the stomach is of caecal type. In *G. chapra* the stomach is voluminous and gizzard like for trituration of coarse plant, sand and/or mud. Such gizzard like stomach was also reported in *T. ilisha* and *Mugil cephalus* by Khanna (1961) which do not have a well formed pharyngeal masticatory apparatus. In the present histological study it has been observed that the gastric mucosa of all the three fish species consists of superficial layer of columnar epithelium. However, the most striking feature of the stomach of *M. cavasius* and *O. niloticus* is the presence of tall columnar epithelial cells justifying its active role of copious amount of mucus secretion which may offer a mucus blanket to protect the underlying epithelial cells from acid secretion

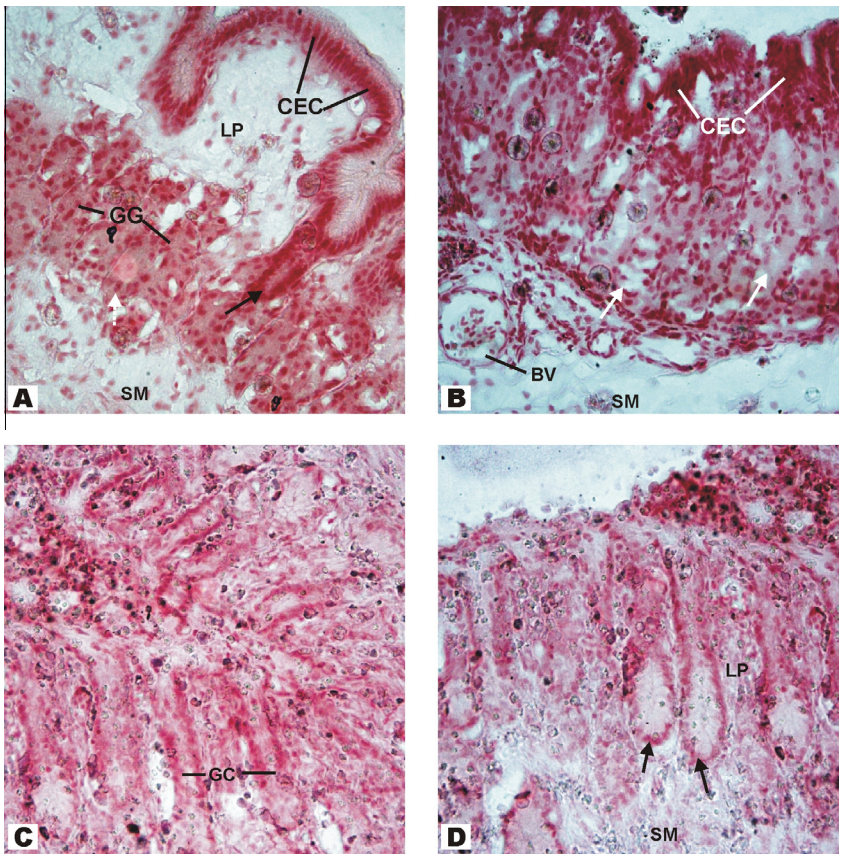

Figure 3 Photomicrographs of section of the stomach of *M. cavasius*, *O. niloticus* and *G. chapra* showing Best's Carmine (BC) reaction for glycogen: (A) maximum glycogen reaction in columnar epithelial cells (CEC) including crypt region (solid arrow) and gastric glands (GG) of the stomach of *M. cavasius*. Note faint reaction in lamina propria (LP) and submucosa (SM) (BC) × 400, (B) showing intense glycogen reaction in CEC, GG (solid arrows) and blood vessels (BV) of the stomach of *O. niloticus*. Note faint reaction in SM (BC) × 400, (C) showing moderate glycogen reaction in the inner lining of the glandular cells (GC) in the stomach of *G. chapra* (BC) × 400, (D) Moderate glycogen reaction in the lining of tubular glands (solid arrows) of *G. chapra*. Note weak reaction in LP and SM (BC) × 400.

and enzyme. On the contrary, the epithelial cells lining the stomach in *G. chapra* are short and stubby which hold considerable amount of mucin film over gastric mucosa to protect epithelial mucosa from mechanical rubbing during trituration of food. Kapoor (1957) and Pasha Kamal (1964) established the mucoid nature of columnar epithelial cells and are believed to contribute mucus and thus protect the surface of the stomach from the mechanical injury. Many authors agreed that the gastric glands in fishes are present either in cardiac or pyloric region (Kapoor, 1957; Pasha Kamal, 1964; Mehrotra and Khanna, 1969). In the present study in *M. cavasius* gastric glands are present only in cardiac portion although a few of them extend in the anterior parts of the pyloric stomach also. Agrawal and Sharma (1966) recorded the tubular gastric glands in the cardiac stomach along with pyloric part in *M. vittatus*. In *O. niloticus* simple tubular gastric glands are present in cardiac part of the stomach whereas in *G. chapra* the gastric glands are absent in the stomach. *M. cavasius* being a carnivore may require rapid secretion of digestive enzymes for the effective digestion of protein food and therefore, gastric glands appear to be more compact and numerous. On the contrary, glandular cells of the stomach of herbivorous *O. niloticus* possess a well developed tubular network. A similar tubular system is a characteristic of mammal parietal cells that are responsible for the stomach's remarkable capacity to secrete hydrochloric acid (Bloom and Fawcett, 1986). Oxynticopeptic cells of fishes also reported by Noaillac-Depeyre and Gas (1978) in *Perca fluviatilis*. Garguillo et al. (1997) mentioned that oxyntic cells are involved in acid production and their secretion may play a role in regulating the pH of the gastric lumen in *Tilapia* sp. Strong prussian blue reaction in the subepithelial portion of the stomach of *O. mossambicus* was noticed by Chakrabarti et al. (1994) and opined the presence of HCl-secreting cells taking part in the secretion of hydrochloric acid for effective break down of algal wall. Acid lysis in the stomach is an indispensable part for the commencing digestion of algae and detrital bacteria in the teleosts (Osman and Caceci, 1991; Chakrabarti et al., 1992). The well developed muscular wall of the stomach of *G. chapra* along with stubby and rugae mucosal epithelium probably maintains a mechanical support to masticate all the food particles which the fish ingests. In *G. chapra* gastric glands are absent which do not have any role of gastric digestion.

The conspicuous columnar epithelial cells lining the mucosal border of the stomach in *M. cavasius*, *O. niloticus* and *G. chapra* discharge their secretion products "mucin" by

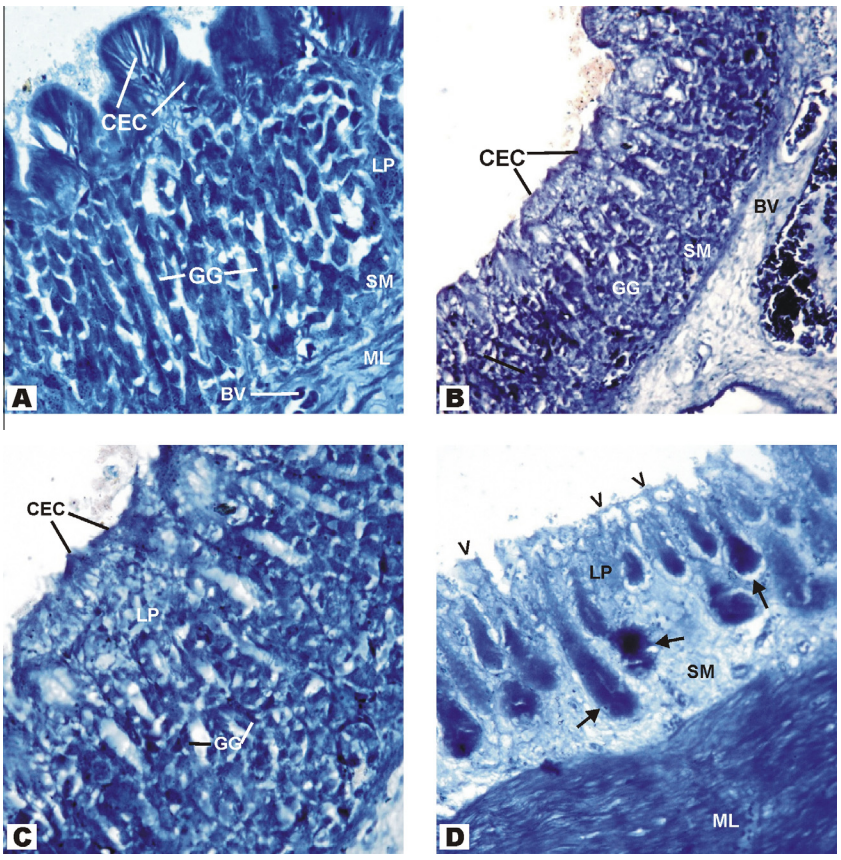

Figure 4 Photomicrographs of section of the stomach of *M. cavasius*, *O. niloticus* and *G. chapra* showing Mercuric Bromophenol Blue (MBPB) reaction for protein: (A) stomach of *M. cavasius* showing intense protein reaction in columnar epithelial cells (CEC), gastric glands (GG) and blood vessels (BV). Note moderate reaction in lamina propria (LP), submucosa (SM) and weak reaction in muscularis layer (ML) (MBPB) × 400, (B) intense reaction of protein in CEC, GG and BV in the stomach of *O. niloticus*. Note moderate reaction in SM (MBPB) × 100, (C) higher magnification of the stomach of *O. niloticus* showing intense protein reaction in CEC and GG. Note weak reaction in LP (MBPB) × 400, (D) stomach of *G. chapra* showing intense protein reaction in tubular glands (solid arrows) and ML. Note weak reaction in CEC, LP and SM (MBPB) × 400.

exocytosis (Murray et al., 1994; Domeneghini et al., 1999; Kazorić et al., 2007). In the present study PAS–AB histochemical test demonstrates that epithelial surface of the columnar epithelial cells is strongly PAS positive and stain weakly with AB in the stomach of *M. cavasius*, *O. niloticus* and *G. chapra*. This unequivocally suggests the predominance of neutral mucin in the epithelial cells along with mucosal border of the stomach of aforesaid fishes. The neutral mucopolysaccharides in the apical portion of epithelial lining in the stomach of *M. cavasius* and *O. niloticus* have a buffering effect and could serve to protect the underlying mucosa layer from the acid environment and proteolysis. The presence of neutral mucosubstances was also observed in the superficial gastric epithelium of the Atlantic bluefin (Grau et al., 1992) and white sturgeon (Domeneghini et al., 1999). On the other hand, the exclusively thick film of neutral mucin observed on the apical surface of the columnar epithelial cells in *G. chapra* is associated with lubrication and conduction of masticated food materials, it also withstands the trauma resulting from ingested materials in order to compensate for the lack of pharyngeal masticatory apparatus. This is in conformity with the findings of Murray et al. (1994) in the stomach of three species of pleuronectids.

The maximum glycogen reaction in the epithelial lining of the stomach of *M. cavasius* and *O. niloticus* may be related to the synthesis and secretion of neutral mucin, which is an active process, requires energy and presence of glycogen which is the main source of energy. Higher reaction of glycogen in the gastric glands of *M. cavasius* and *O. niloticus* is related to the synthesis of zymogen granules and ergastic substances in the production of pepsinogen and secretion of HCl in the aforesaid fishes. The moderate reaction of glycogen in the lining of tubular glands of *G. chapra* is probably concerned with the formation of neutral mucopolysaccharides. Neutral glycoproteins and ATPase activity present in the stomach mucosa of *Solea senegalensis* could also be involved in the synthesis of neutral glycoconjugates (Gisbert et al., 1999).

The maximum reaction for protein and tryptophan in the gastric glands of *M. cavasius* may be related to their high zymogen content as a precursor of pepsinogen. Medeiros et al. (1970) identified arginine, tryptophan and tyrosine from glandular cells of the stomach in *Pimelodus maculates*. The

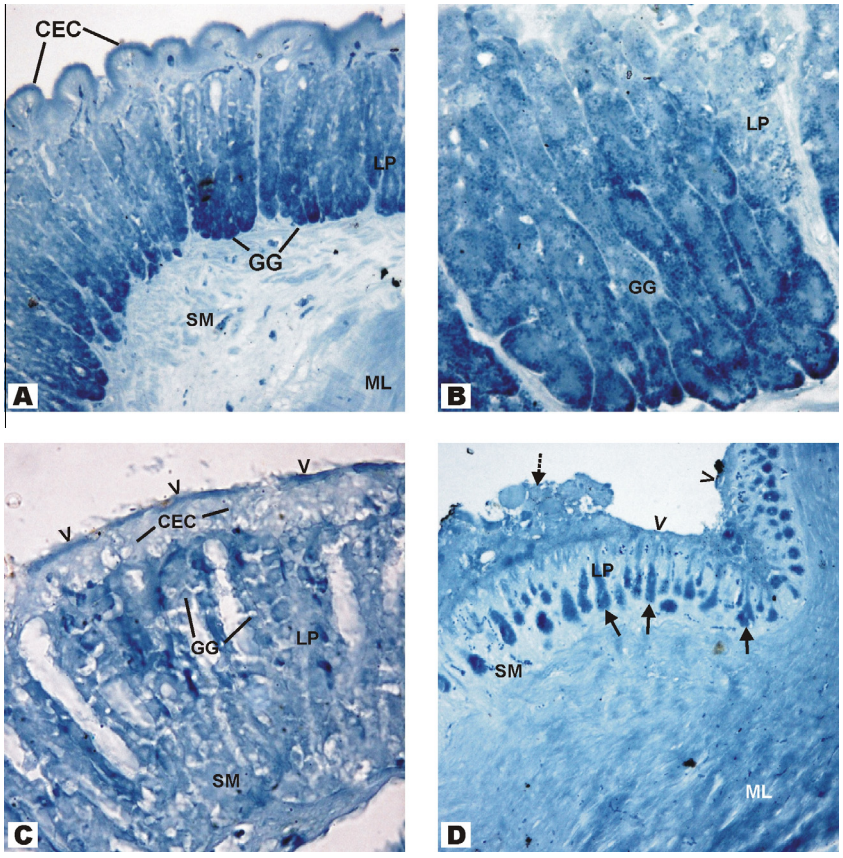

Figure 5 Photomicrographs of section of the stomach of *M. cavasius*, *O. niloticus* and *G. chapra* showing histochemical localization of tryptophan (DMAB): (A) stomach of *M. cavasius* showing intense tryptophan reaction in gastric glands (GG), moderate reaction in columnar epithelial cells (CEC) and weak reaction in submucosa (SM) and muscularis layer (ML) (DMAB) × 200, (B) higher magnification of GG in *M. cavasius* showing intense tryptophan reaction as granular deposition in gastric cells. Note weak reaction in LP (DMAB) × 400, (C) intense tryptophan reaction in mucosal border (arrow heads) and GG of the stomach of *O. niloticus*. Note feeble reaction in CEC, LP and SM (DMAB) × 400, (D) stomach of *G. chapra* showing positive tryptophan reaction in the tubular glands (solid arrows). Note weak reaction in SM, ML, luminal border (arrow heads) and luminal secretion (broken arrow) (DMAB) × 100.

localization of varying amounts of protein and tryptophan in the columnar epithelial cells as well as in the tubular glands of *G. chapra*, *M. cavasius* and *O. niloticus* may be related for metabolic activity of the cell concerned.

Conclusion

In conclusion the stomach of *M. cavasius*, *O. niloticus* and *G. chapra* has revealed many variations that are undoubtedly correlated with their different feeding habits. The columnar epithelial cells lining the gastric mucosa of all the three fish species secrete neutral mucin justifying their active role to protect the underlying epithelial cells from acid, enzymes and mechanical rubbing. Positive reaction of glycogen, protein and tryptophan in the gastric glands of *M. cavasius* and *O. niloticus* is related to the synthesis of ergastic substances and zymogen granules as the precursor of gastric enzymes and/or hydrochloric acid respectively. The moderate reaction of glycogen, protein and tryptophan in the tubular glands of G. chapra may be concerned with formation of neutral mucopolysaccharides and also related to metabolic activity of the cell concerned. However, the results presented in the current study may be considered as a base line for subsequent studies on the stomach of different teleosts.

Acknowledgement

The authors are grateful to Dr. A. Basu, Head of the Department of Zoology, The University of Burdwan, Burdwan for providing necessary laboratory facilities.

References

Adams, C.W.M., 1957. A p-Dimethylaminobenzaldehyde nitrate method for the histochemical demonstration of tryptophan and related compounds. J. Clin. Pathol. 10, 56–62.

Agrawal, V.P., Sharma, U., 1966. Morpho-histological studies of the digestive tract of *Mystus vittatus* (Bloch). Proc. Natl. Acad. Sci. 36, 441–456.

Anderson, T.A., 1986. Histological and cytological structure of the gastrointestinal tract of the luderick, *Girella tricuspidata*, in relation to diet. J. Morphol. 190, 109–119.

Arellano, J.M., Storch, V., Sarasquete, C., 2001. Histological and histochemical observations in the stomach of the Senegal sole, *Solea senegalensis*. Histol. Histopathol. 16, 511–521.

Banan Khojasteh, S.M., 2012. The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. Int. J. Aquat. Sci. 3, 71–88.

Best, F., 1906. Uber carmine far bug des glycogens and derkerne. Z. Wiss. Mikr. 3, 319–322.

Bloom, W., Fawcett, D.W., 1986. A Text Book of Histology, 10th ed. W.B. Saunders, Philadelphia.

Buddington, R.K., Krogdahl, Å., Bakke-Mc Kellep, A.M., 1997. The intestines of carnivorous fish: structure and function and the relations with diet. Acta Physiol. Scand. 161, 67–80.

Carrassón, M., Grau, A., Dopazo, L.R., Crespo, S., 2006. A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). Histol. Histopathol. 21, 579–593.

Chakrabarti, P., Mandal, D.K., Ganguly, S., 1992. A scanning electron microscope study of the mucosal epithelium of the alimentary canal of stomach bearing herbivorous fish *Oreochromis mossambicus* (Peters). Eur. Arch. Biol. 103, 265–270.

Chakrabarti, P., Ganguly, S., Mandal, D.K., 1994. Histochemical studies of the digestive system of freshwater cichlid fish *Oreochromis mossambicus* (Peters). J. Freshwater Biol. 6, 63–69.

Diaz, A.O., Garcia, A.M., Devincenti, C.V., Goldemberg, A.L., 2003. Morphological and histochemical characterization of the mucosa of the digestive tract in *Engraulis anchoita* (Hubbs and Marini, 1995). Anat. Histol. Embryol. 32, 341–346.

Domeneghini, C., Arrighi, S., Radaelli, G., Bosi, G., Mascarello, G.S., 1999. Morphological and histochemical peculiarities of the gut in white sturgeon, *Acipenser transmontanus*. Eur. Histochem. 43, 135–145.

Gallagher, M.L., Luczkovich, J.J., Stellwag, E.J., 2001. Characterization of the ultrastructure of the gastrointestinal tract mucosa, stomach contents and liver enzyme activity of the pinfish during development. J. Fish Biol. 58, 1704–1713.

Gargillo, A.M., Ceccarelli, P., Dall'aglio, C., Pedini, V., 1997. Ultrastructural study on the stomach of *Tilapia* spp (Teleostei). Anat. Histol. Embryol. 26, 331–336.

Gisbert, E., Sarasquete, M.C., Williot, P., Castelló-Orvay, F., 1999. Histochemistry of the development of the digestive system of Siberian sturgeon during early ontogeny. J. Fish Biol. 55, 596–616.

Gona, O., 1979. Mucous glycoproteins of teleostean fish. A comparative histochemical study. J. Histochem. 11, 709–718.

Grau, A., Crespo, S., Sarasquete, M.C., Gonzalez de Canales, M.L., 1992. The digestive tract of the amberjack *Seriola dumerili*, Risso: a light and scanning electron microscope study. J. Fish Biol. 41, 287–303.

Ikpegbu, E., Ezeasor, D.N., Nlebedum, U.C., Nnadozie, O., 2013. Morphological and histochemical observations on the oesogaster of the domesticated African catfish (*Clarias gariepinus*, Burchell, 1822). B.J.V.M. 16, 88–95.

Kapoor, B.G., 1957. The morphology and histology of alimentary tract of a plankton-feeder, *Gudusia chapra* (Hamilton). Ann. Mus. Civ. Stor. Nat. Gen. 70, 8–32.

Kapoor, B.G., Smit, H., Verighina, A., 1975. The alimentary canal and digestion in teleosts. Adv. Mar. Biol. 13, 109–239.

Kozaric, Z., Kuzir, S., Petrinec, Z., Gjurcevic, E., Baturina, N., 2007. Histochemistry of complex glycoproteins in the digestive tract mucosa of Atlantic bluefin tuna (*Thunnus thynnus*) L. Vet. Arh. 77, 441–452.

Khalaf Allah, H.M.M., 2013. Morphological adaptations of digestive tract according to food and feeding habits of the broomtail wrasse, *Cheilinus lunulatus*. Egypt. J. Aquat. Biol. Fish 17, 123–141.

Khanna, S.S., 1961. Alimentary canal in some teleost fishes. J. Zool. Soc. India 113, 206–219.

Mallory, F.B., 1936. The aniline blue collagen stain. Stain Technol. 11, 101.

Mazia, D., Brewer, P.A., Alfert, M., 1953. The cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol. Bull. 104, 57.

Medeiros, L.O., Ferris, S., Godinha, H., Medeiros, L.F., 1970. Proteins and polysaccharides of the club shaped cells in the lining epithelium of fish (*Pimelodus maculates*) digestive tract: histochemical study. Ann. d'Histochim. 15, 181–186.

Mehrotra, D.K., Khanna, S.S., 1969. Histomorphology of the oesophagus and the stomach in some Indian teleosts with inference on their adaptational features. Zool. Beitraege Berl. 15, 375–391.

Mowry, R.W., 1956. Alcian blue technique for the histochemical study of acidic carbohydrates. J. Histochem. Cytochem. 4, 403.

Murray, H.M., Wright, G.M., Goff, G.P., 1994. A comparative histological and histochemical study of the stomach from three species of pleuronectid, the Atlantic halibut, *Hippoglossus hippoglossus*, the yellowtail flounder, *Pleuronectes ferruginea*, and the winter flounder, *Pleuronectes americanus*. Can. J. Zool. 72, 1199–1210.

Murray, H.M., Wright, G.M., Goff, G.P., 1996. A comparative histological and histochemical study of the post-gastric alimentary canal from three species of pleuronectids, the Atlantic halibut, the Yellowtail flounder and the Winter flounder. J. Fish Biol. 48, 187–206.

Noaillac-Depeyre, J., Gas, N., 1978. Ultrastructural and cytochemical study of the gastric epithelium in a freshwater teleostean fish (*Perca fluviatilis*). Tissue Cell 10, 23–37.

Osman, A.H.K., Caceci, T., 1991. Histology of the stomach of *Tilapia nilotica* (Linnaues, 1758) from the river Nile. J. Fish Biol. 38, 211–223.

Pasha Kamal, S.M., 1964. Anatomy and histology of the alimentary canal of a herbivorous fish, *Tilapia mossambica*. Proc. Ind. Acad. Sci. 59B, 340–349.

Pillay, T.V.R., 1953. Studies on the food, feeding habits and alimentary tract of grey mullet, *Mugil tade*. Proc. Nat. Inst. Sci. 19, 777–827.

Rebolledo, I.M., Vial, J.D., 1979. Fine structure of the oxynticopeptic cells in the gastric glands of an elasmobranch species (*Halaerurus chilensis*). Anat. Rec. 193, 805–821.

Reifel, C.W., Travill, A.A., 1978. Structure and carbohydrate histochemistry of the stomach in eight species of teleosts. J. Morphol. 158, 155–167.

Winemiller, K.O., Kelso-Winemillar, L.C., Brenkert, A.L., 1995. Ecomorphological diversification and convergence in fluvial cichlid fishes. Environ. Biol. Fish 44, 235–261.